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SELECTION OF POTENTIAL PROBIOTIC BACTERIA FROM THE INTESTINES OF LETHRINUS CONCHYLIATUS AND OREOCHROMIS MOSSAMBICUS PRODUCING BACTERIOCIN AND ITS APPLICATION AS A BIOPRESERVATIVE

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ABSTRACT

Probiotic are non-pathogenic, low-GC containing, acid-tolerant, and Generally Recognized As Safe (GRAS) microorganisms. Viability and metabolic activities are important characteristics of probiotic microorganisms. They secrete substances that inhibit pathogenic bacteria which is known as BLIS (Bacteriocin Like Inhibitory Substance). Probiotics includes the genera *Lactobacillus, Bifidobacterium, Pediococcus* etc. The present work explores the isolation of potential probiotic from GIT of marine *Lethrinus conchyliatus* (Emperor Fish) and fresh water fish *Oreochromis mossambicus* (Tilapia), Probiotic characteristics (acid, bile salt and salt tolerant), growth in varied temperature, antibiotic profile and screening of bacteriocin producing organisms. We extracted and partially purified the bacteriocin by ammonium precipitation and dialysis method. *Lactobacillus* sp. (EF2) showed the best results in bacteriocin production. The Partially Purified Bacteriocin (PPB) was analysed for its preservative capability on the raw fish as bacteriocin would be a future organic preservator in food industries.

INTRODUCTION

The concept of probiotics was first proposed by Elie Metchnikoff, a Noble Laureate of the year 1908. FAO/WHO defined it more precisely as-live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2002). Certain strains of bacteria have been discovered over the years to have probiotic properties, mainly consisting of lactic acid producing bacteria (*Lactobacilli, Streptococci, Enterococci, Lactococci, Bifidobacteria*), *Bacillus* and yeast *Saccharomyces* and fungi such as *Aspergillus*.

The probiotic microorganisms should not be pathogenic, have no connection with diarrhoeagenic bacteria and no ability to transfer antibiotic resistance genes, as well as they able to maintain genetic stability. To be recognized as functional food components, they should demonstrate the following properties viz., acid- and bile-stability, resistance to digestive enzymes, adhesion to intestine surface, antagonistic activity against human pathogens, anti-carcinogenic and anti-mutagenic activity, cholesterol-lowering effects, stimulation of the immune system without inflammatory effects, enhancement of bowel motility, maintenance of mucosal integrity, improvement of bioavailability of food compounds and production of vitamins and enzymes (Galina *et al*., 2012**)** The technological properties of bacteria play a very significant role in the production of bacteriocin (Saarela *et al*., 2000).

Bacteriocin is a protein peptide with low molecular weight and considered as safe natural preservatives or biopreservatives, as they can be degraded by the proteases in the gastrointestinal tract. Cleveland *et al*., 2001. The bacteriocins produced by probiotic microorganisms are Generally Recognized As Safe (GRAS) substances which are not active and nontoxic and become inactivated by digestive proteases which are usually pH and Heat -tolerance. They show a bactericidal mode of action, usually acting on the bacterial cytoplasmic membrane: no cross resistances with antibiotics and their genetic determinants are usually plasmid- encoded, facilitating manipulation (Onwuakor, 2014).

The shift to more ‘‘natural’’ foods has resulted in a great interest in the use of bacteriocins from lactic acid bacteria as natural biopreservatives. Select bacteriocin producers were cell-free supernatants (CFS) were frozen, and the frozen CFS

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samples were freeze-dried to produce bacteriocin-containing powders (Galina et al., 2012). However, only the nisin has been approved as a food additive and can be used in various foods (Delves-Broughton *et al*., 2005). In this present study to check the bacteriocin activity towards the fresh water fish *Oreochromis mossambicus* (Tilapia) to increase the shelf life was performed and calculated. As the bacteriocin can be used as preservative this is produced by the isolate EF2.

MATERIALS AND METHOD

Sample Collection

Selected marine water fish *Lethrinus conchyliatus* (Emperor Fish) and fresh water fish

Oreochromis mossambicus (Tilapia) were purchased from local fish market, Dindigul, Tamil Nadu, India. These samples were transferred to GRI laboratory in sterile condition through sterile container.

Isolation of bacteria from fish gastro instestinal tract (gut)

Fish samples were washed, dissected and transferred to the enriched broth media. Dilutions of 10^{-5} and 10^{-6} were plated on to De Man Rogosa and Sharpe agar (Hi Media, India), Nutrient agar medium(Hi Media, India) and incubated at 31-32^{0}C aerobically in incubator and anaerobically in Anaerobic jar for 48 hours.

Identification of bacteria

The isolated bacteria Colonies which have a clear halo zones were randomly selected. Totally 23 strains were isolated, among them 7 strains were selected and the colony morphology, Gram positive, catalase-negative and carbohydrate fermentation was performed. Among them the three strains identified as *Lactobacillus* sp. (EF 2) and *Lactococcus* sp. (TF1 and TF5) were stored at -20ºC in MRS broth supplemented with 50% (v/v) glycerol and subcultured twice in MRS broth (Hi Media, India)for periodic analysis.

Evaluation of Probiotic Property

Acidification and Coagulation

All the isolates were analyzed for the acidification and coagulation properties by inoculating 10% of sterile skim milk (Hi Media, India) with Lab strains. Observation was made for the commencement of Clotting and Odour and pH was checked after 24 hours of incubation 37 ±2°C (Olassupo *et al*., 2001).

Resistance to Bile

Fresh MRS broth was prepared with different concentration of bile viz., 0.1%, 0.3%, and 0.5%, broth without bile as control. MRS broth was inoculated with cultures containing 10^6 cells/ml in to each concentration and incubated. 0.1 ml of culture from the different concentration was spread over the prepared MRS agar medium plates for subsequent 0th, 3rd and 5th hours and incubated at 37±2^O C for 24 hours and survival was evaluated by plate count technique.

Resistance to Acid

The isolated strains EF2, TF1 and TF5 were tested for the tolerance to Acidic. Different pH viz., pH 2, pH 3 and pH 7(Control) were maintained in MRS medium and 1 ml of three strains were inoculated to the series of test tubes containing MRS medium with different pH, 0. 1 ml of culture from different tubes were plated for 0th hour, 3rd hour and 5th hour and the plates were incubated for 24 hours and the results were calculated by following formula.

$$\% \text{ survival} = \frac{\text{3hr survival count of LAB (log CFU/ ml)}}{\text{Initial count of LAB (log CFU/ ml)}} \times 100$$

Cell surface hydrophobicity test

The adherence ability of isolated strains to intestinal epithelial cells was analyzed by this test. Cell Free Culture Supernatants (CFCS) were obtained by centrifugation (1000× g, 4°C) for 20 min. The pellet was washed twice with phosphate buffer. OD value of 0.5 was maintained at 600 nm, 1 ml of toluene followed by 3ml of washed cells were added. This mixture was blended with vortex for 90 seconds. Tubes were left for 15 min for the separation of two phases and the OD of aqueous phase was measured at 600 nm using the spectrophotometer.

$$h\% = \frac{\text{OD of 600 before mixing} - \text{OD of 600 after mixing}}{\text{OD of 600 before mixing}} \times 100$$

Determination of growth at various external factors

The ability of isolated strains to grow in the presence of various external factors such as pH, NaCl and temperature. The isolates were inoculated into the broth containing various pH viz., 3, 7, and 9 and the temperature such as 4°C, 20°C and 37°C and various concentration of NaCl viz., 0.3%, 0.5% and 1.0% . The inoculated broth was incubated for 24 hours at 37 ± 2°C. The results were recorded using spectrophotometer (Spectronic 200) at 600 nm.

Antibiotic resistance profile

Antibiotic susceptibility patterns were determined by the disk diffusion method (Bauer *et al*., 1996). Isolated strains were swabbed over the prepared MRS agar plates and prepared antibiotic-containing disks were overlaid on MRS agar previously the antibiotics were dissolved in 0.5µl of sterile water. The antibiotics used were Ampicillin (20µg), Erythromycin (20µg), Amoxicillin (20µg), Metronidazole (20µg), Penicillin (20µg), Tetracycline (20µg) and Streptomycin (20µg). Inhibition zone diameters were measured after overnight incubation at 37°C.

Antimicrobial spectrum of Bacteriocin

Bacteriocin production were identified by *Escherichia coli* MTCC 2622, *Staphylococcus aureus* MTCC 7278, *Pseudomonas aeruginosa* MTCC 741, *Klebsiella Pneumoniae* MTCC 7048, *Enterococcus aureus* MTCC 439, *Candida albicans* MTCC 8017, *Candida tropicalis* MTCC 184,

Bacillus cereus MTCC 7278*, Listeria monocytogenes* MTCC 657 were examined by agar well-diffusion method. Briefly, the probiotic strains to be tested were inoculated into 50 ml MRS broth and incubated overnight for BLIS production. CFCS were obtained by centrifugation (1000× g 4°C, 20 minutes) and filtered through 0.22 µm membrane filter (Millipore, USA) to remove residual bacterial cells. To test the BLIS activity of CFCS, the indicator organism was spread onto nutrient agar

plate and 6 mm- diameter well were punched into the surface using a sterile cork borer. Consequently, 100µl of pH neutralized CFCS was added to each well and incubated at optimal growth condition required for pathogens. The antimicrobial activity recorded as growth free inhibition zones (diameter) around the well. Fraction showing antimicrobial activity was considered as BLIS. The BLIS activity calculated using following equation.

BLIS (mm^2/ml)=(LZ-LS/Volume of sample) here mm power 2

Where, Lz = clear zone area (mm^2), Ls = well area (mm^2), V = volume of sample (ml).

Partial purification of bioactive substance

To partially purify the bioactive protein (bacteriocin) was precipitated with 40% (by mass per volume) Ammonium Sulphate (AS) and stirred overnight at 4°C. Then the precipitated proteins was collected by centrifugation at 1000×g for 20 min at 4°C and dissolved in phosphate buffer saline (PBS) at pH 7.4 resulting 40% precipitate were dialyzed using a dialyzing bag (10 KDa) for 16 hours against the same buffer at 4°C. The presence of proteinaceous substance in dialysate fraction was confirmed by performing agar well diffusion assay.

Biopreservative effect of bacteriocin on refrigerated seafood

To determine the inhibitory action of bacteriocin against spoilage microorganism (Li-Jung Yin *et al*., 2007) was performed. The fish was thawed at -21^{o}C during day time 4^{o}c for overnight. Then it was dissected aseptically into 3-5 gm pieces. Sliced samples were immersed in crude and partially purified bacteriocins for 3 min. Control sliced were immersed in 0.02 N Hcl without bacteriocin. All the samples were drained and grinded with 90 ml of sterilized 0.1% peptone water. 1 ml of this mixture was serially diluted the dilutions of $10^{-2,}$ $10^{-3,}$ $10^{-4,}$$10^{-5}$ and 10^{-6} were dispensed in Nutrient agar medium by pour plate method and the plates were incubated at 25^{o}c for 24 hours. The colonies (CFU) were calculated this procedure was respected for a week (0, 1, 3, 5 &7 days) by storing the dissected sample aseptically in refrigerated at 4^{O}C.

RESULTS AND DISCUSSION

Enumeration of bacteria from samples

Lactic acid bacteria are an extremely important group of probiotic bacteria inhibit undesirable microflora in the gut and create a healthy equilibrium between beneficial and potentially intestinal pathogens (Tambekar *et al*., 2010). In this study, we enumerated (Table 1), isolated, identified and characterized Lactic acid bacteria from selected marine water fish *Lethrinus conchyliatus* (Emperor Fish) and fresh water fish *Oreochromis mossambicus* (Tilapia). We isolated 23 strains and selected 7 strains based on their clear halo zone formation and their morphological studies were carried out (Table 2).

We evaluated various probiotic properties for a group of bacteria from selected marine water fish *Lethrinus conchyliatus* (Emperor Fish) and fresh water fish *Oreochromis mossambicus* (Tilapia). Among them three gram positive strains viz., EF2, TF1 and TF5. The EF2 and TF1 strains show positive results Methyl red test, Carbohydrate fermentation test

(Table 3) and Starch hydrolysis test and catalase negative on TF5 strains (Table 4). Based on their morphological and biochemical characteristics, EF2 was conformed as *Lactobacillus* sp; TF1 and TF5 *Lactococcus* sp. (Ghanbari *et al*., 2009) evaluated and biochemical characteristics of 84 strains isolated from intestines of beluga and Persian sturgeon of *Lactobacillus* sp.

Adequate numbers of probiotic viable cells should reach the intestinal tract to exert its beneficial effects in order to survive the passage through GIT resistant to low pH and bile salt is necessary. Acid and bile tolerance is considered as the most important properties (Fig1 &2) of probiotic microorganism. pH 2, pH 3 and pH 7 was tested for all the three strains. Among them TF1 *Lactococcus* sp. strains exhibited high survival rate at pH 3 compares to other two strains. EF2 and TF5 showed minimum survival rate in pH 3. Similarly, Chou and Weimer, isolated acid and bile resistant variants of *Lactobacillus* sp. some of these strains were found to be resistant to acid a pH 3.5 for 90 min at 37± 2^{o}C. These strains were capable of growth in medium at pH 3.5(Liong and Shah, 2005 and Gilliland, 1977).

Table 1 Enumeration of Lactic Acid Bacteria (LAB)

S.NO	Samples	Incubation	Dilution factor	Number of colonies			Log CFU/ml
				Duplicate 1	Duplicate 2	Average	
1	*Lethrinus conchyliatus* (Emperor fish)	Aerobic	10^{-5}	110	90	100	5.78
			10^{-6}	84	60	72	
2	*Lethrinus conchyliatus (Emperor fish)*	Anaerobic	10^{-5}	269	221	245	5.16
			10^{-6}	196	134	165	
3	*Oreochromis mossambicus* (Tilapia)	Aerobic	$_{10}$-5	162	115	139	5.11
			10^{-6}	120	97	109	
4	*Oreochromis mossambicus* (Tilapia)	Anaerobic	10^{-5}	306	298	302	5.26
			$_{10}$-6	254	292	273	

Table 2 Morphological Characteristics of Bacterial Isolates

S.No	Bacterial Isolates	Colony shape	Edges	Elevation	Surface	Pigmentation	Gram's Reaction	Catalase Test	Shape	Motility	Curdling property
1	EF1	Irregular	Undulate	Raised	Wrinkled	No pigmentation	Positive	Positive	Rod	Non motile	Odourless
2	EF2	Circular	Entire	Flat	Smooth	No pigmentation	Positive	Negative	Rod	Non motile	Perfect odour
3	TF1	Irregular	Dulate	Flat	Wrinkled	No pigmentation	Positive	Negative	Cocci	Non motile	Perfect odour
4	TF2	Irregular	Dulate	Flat	Wrinkled	No pigmentation	Positive	Positive	Rod	Non motile	Odourless
5	TF3	Irregular	Dulate	Flat	Wrinkled	No pigmentation	Positive	Positive	Cocci	Non motile	Odourless
6	TF4	Circular	Undulate	Flat	Smooth	No pigmentation	Positive	Positive	Cocci	Non motile	Odourless
7	TF5	Circular	Lobed	Raised	Smooth	No pigmentation	Positive	Negative	Cocci	Non motile	Odourless

Table 3 Carbohydrate Fermentation Test

S.NO	Sugars	Bacterial isolates					
		EF2		TF1		TF5	
		Acid	Gas	Acid	Gas	Acid	Gas
1	Glucose	+	-	+	-	+	-
2	Fructose	+	-	+	-	+	-
3	Sucrose	+	-	+	-	-	-
4	Lactose	+	-	+	-	+	-
5	Mannitol	+	-	+	-	+	-
6	Maltose	+	-	+	-	+	-
7	Arabinose	+	-	+	-	+	-
8	Sorbitol	+	-	+	-	+	-
9	Xylose	+	-	+	-	+	-

Table 4 Biochemical Characterization of Bacterial Isolates

S.NO	Biochemical test	Bacterial Isolates		
		EF2	TF1	TF5
1	Indole production	-	-	-
2	Methyl red test	+	-	-
3	Voges proskauer test	-	-	-
4	Catalase activity	-	-	-
5	Citrate utilization test	-	-	-
6	Starch hydrolysis test	+	+	-
7	Gelatin hydrolysis test	-	-	-
8	Triple sugar Ion test	-	-	-
9	Genus	*Lactobacillus* sp.	*Lactococcus* sp.	*Lactococcus* sp.

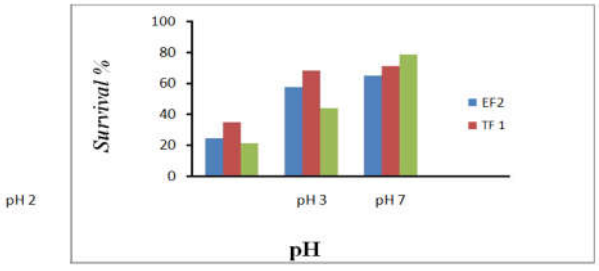

Figure 1 Resistance to Acid

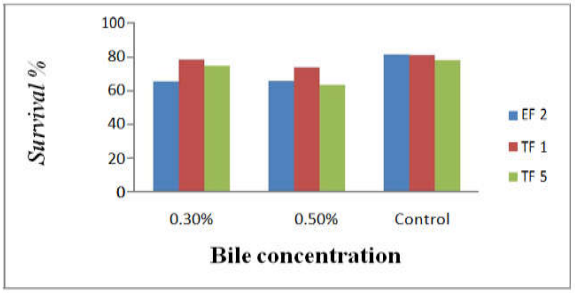

Figure 2 Resiatance to Bile

Each value in Figure 1 and 2 is the total plate count log 10 CFU/ ml of three independent Experiments the ability of isolated strains to the adhesion of microorganisms to intestinal epithelial cells tested by hydrophobicity method. In our study adhesion of three strains *Lactobacillus* sp. EF2, *Lactococcus* sp. TF1 and TF5 were tested used. Among them, EF2 strain was more adhesion compare to other two strains (Table 5). A higher percentage of hydrophobicity has been observed due to the physiochemical properties of the microbial cell surface which have unfolded glycoprotein materials (Kos *et al*., 2003).

The bacterial growth (EF2, TF1 & TF5) at different NaCl concentration, pH and temperature. The bacterial isolates EF2, TF1 and TF5 have ability to grow at 0.1%, 0.3% and 0.5 of NaCl concentration, pH 3, pH 7, and pH 10 and temperature 4^{o}C, 20 ^{o}C, 37 ^{o}C. were read at 600nm and Tabulated as Table 6

Table 5 Adherence Capacity

S. No	Strains	Adherence (%)
1	EF2	89.03
2	TF1	87.28
3	TF5	73.35

Table 6 Growth of Bacterial Isolates at Different NaCl, pH and Temperature

S.NO	Bacterial isolates	Absorbance and OD value of 600 nm								
		NaCl concentration			pH			Temperature (^{o}C)		
		0.1	0.3	0.5	3	7	10	4	20	37
1	EF2	0.126	0.317	0.126	0.157	0.249	1.02	0.059	0.196	0.26
2	TF1	0.324	0.254	0.389	0.077	0.793	0.098	0.062	0.175	0.29
3	TF5	0.276	0.229	0.136	0.021	0.379	0.817	0.042	0.209	0.27

Antibiotics used for treating animals can enhance the development of antibiotic resistant in intestinal microflora. These antibiotic resistant bacteria can transfer the resistant substance to other pathogenic bacteria by alternating the genetic material (Dima, 2014). In our study, the selected *Lactobacillus* sp. EF2, and TF1 were resistant to two antibiotics viz., Amoxicillin and Ampicillin and susceptible to Erythromycin, Penicillin, Tetracyclines Streptomycin and Metronidazole. TF5 were resistant to Erythromycin and Tetracycline and susceptible to Penicillin, Streptomycin, Metronidazole, Amoxicillin and Ampicillin (Table 7). Rudresh *et al*., 2010 reported that the strain lactic acid bacteria (LAB) isolates obtained from fish

Table 7 Antibiotic Sensitivity Test

S.NO	Antibiotic	EF2	TF1	TF5
1	Erythromycin (20µg)	0.00	0.00	2.46±0.31
2	Penicillin (20µg)	0.00	0.00	0.00
3	Amoxicillin (20µg)	1.96 ± 0.41	2.13 ± 0.21	0.00
4	Tetracycline (20µg)	0.00	0.00	1.83 ± 0.35
5	Ampicillin (20µg)	2.1 ± 0.4	1.33 ± 0.15	0.00
6	Streptomycin (20µg)	0.00	0.00	0.00
7	Metronidazole (20µg)	0.00	0.00	0.00

Each value in Table 7 is the means ± standard deviations of three independent experiments

Bacteriocin Production is one of the most important selection criteria for probiotics. Bacteriocin targets the enteric undesirables and pathogens. Probiotic Lactobacilli strains produced antimicrobial substances against pathogens. (Klaenhammer 1999). The supernatants of *Lactobacillus* sp. EF2, *Lactococcus* sp. TF1 and TF5 produced clear zone against the test organism. *Lactobacillus* sp. EF2 shows *Escherichia coli* MTCC 2622 1355.88 compared to other strains. TF1 with the maximum zone of 1770.88 against *Pseudomonas aeruginosa* and TF5 show the maximum zone of 994 *Enterococcus faecalis*. Antimicrobial metabolites produced by the isolates inhibited growth of the pathogens and produced respective zones of inhibition (Table 8). The isolates showed moderate to high levels of inhibition of all five indicator strains. The inhibition spectrum against *Escherichia coli* and *Salmonella* sp. (Aparna balakrishna *et al*., 2012)

bacterial species. This method applied to preserve the seafood by using bacteriocin to inhibit the growth of spoilage causing bacteria (Anil *et al*., 2017). In our study, *Lactobacillus* sp., EF2 strains was used as preservative, the crude bacteriocin controlled the microbial growth compared to the partial purification of BLIS because the crude bacteriocin contains amino acid, organic acid and enzymes which inhibits, the growth of the spoilage causing microbes in the fish refrigerated seafood and used pediocin ACCEL and nisin bacteriocin to suppress the growth of inoculated *Listeria monocytogenes* during 2- and 1-week storage at 4°C, respectively. Compared with nisin, the pediocin ACCEL was considered to be more effective on the suppression of *L. monocytogenes* growth in refrigerated seafoods during 2-week storage at 4°C (Fig 3). (Li-Jung YIN *et al*., 2007).

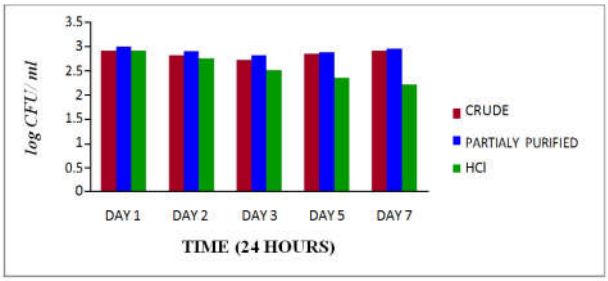

Figure 3 Bacteriocin as food preservative in fish

Each value in Figure 3 is the total plate count log 10 CFU/ ml.

Table 8 Antimicrobial Activity of Crude Bacteriocin against Pathogens

Bacterial isolates	EF2	AU	TF1	AU	TF5	AU
Escherichia coli MTCC 2622	11.67±0.57	1355.88804	9±1	804	8.33±0.57	687.88
Staphylococcus aureus MTCC 7278	11.33±0.57	1277.68	12±1	1434	7.66±0.57	580.75
Pseudomonas aeruginosa MTCC741	10.33±0.57	1061.08	13.33±0.57	1770.88	9.33±2.08	864.48
Klebsiella Pneumoniae MTCC 7048	11±0.57	1204	10.33±0.57	1061.08	8.66±1.15	743.95
*Enterococcus faecalis*MTCC 439	8.66±1.52	743.95	10±1	994	10±1	994
Candida albicans MTCC 8017	10±1	994	10.33±0.577	1061.08	8.66±1.52	743.95
Candida tropicalis MTCC 184	8.66±0.57	743.95	12±1	1434	8.66±2.08	743.95
Bacillus cereus MTCC 7278	8.33±1.15	687.88	10.33±2.51	1061.08	9.33±1.15	864.48
Listeria monocytogenes MTCC 657	10.33±0.57	1061.08	10.33±2.08	1061.08	8.33±1.15	743.95

Each value in Table 8 is the means and standard deviations of independent experiments.

Table 9 Comparision of inhibitory action between CFCS AND PPB OF EF2

Bacterial isolates	CFCS		PPB		% difference in the inhibition zone size	
	EF2	AU	EF2	AU	EF2	AU
Escherichia coli MTCC 2622	11.67±0.57	1355.88804	-	-	-	-
Staphylococcus aureus MTCC 7278	11.33±0.57	1277.68	11.40±0.12	939.60	0.62	27.71
Pseudomonas aeruginosa MTCC741	10.33±0.57	1061.08	11.00±0.81	850	6.57	17.13
Klebsiella Pneumoniae MTCC 7048	11±0.57	1204	12.49±0.37	1200	13.54	36.08
*Enterococcus faecalis*MTCC 439	8.66±1.52	743.95	9.62±0.15	565.44	11.17	10.73
Candida albicans MTCC 8017	10±1	994	12.49±0.37	1200	24.9	31.10
Candida tropicalis MTCC 184	8.66±0.57	743.95	9.18±0.18	482.72	6.00	21.47
Bacillus cereus MTCC 7278	8.33±1.15	687.88	-	-	-	-
Listeria monocytogenes MTCC 657	10.33±0.57	1061.08	12.59±0.37	1225.40	7.97	29.77

Each value in Table 9 is the means and standard deviations of independent experiments.

There is an increased in bacteriocin after partial purification of crude bacteriocin by 40% ammonium sulphate precipitation. The bacteriocin produced by *Lactococcus plantarum* was found to be very effective against both gram positive and gram negative pathogens (Table 9). (Dhanapathi *et al*., 2008).

Bacteriocins are compounds produced by bacteria that exhibit a bactericidal or bacteriostatic mode of action against sensitive

CONCLUSION

Seven strains were isolated from the GIT of marine water fish *Lethrinus conchyliatus* (Emperor fish) and fresh water fish *Oreochromis mossambicus* (Tilapia) and out of that, three strains were selected for further studies based upon their Gram's staining results and catalase test. These organisms showed Gram positive and catalase negative and these strains

were identified as *Lactobacillus* sp. (EF2) and *Lactococcus* sp. (TF1 and TF5) by biochemical test and morphology various probiotic criteria test viz., acid tolerance, bile tolerance, hydrophobicity, and antibiotic susceptibility tests were carried out to select the potential probiotic strains. *Lactobacillus* sp. EF2 strain had shown maximum tolerance towards acidity pH 3 and maximum tolerance to bile of 0.3% and 0.5% concentration. All the three strains were subjected to antibiotic susceptibility test, *Lactobacillus* sp. EF2 were resistant to two antibiotics viz., Amoxicillin and Ampicillin and susceptible for five antibiotics. Antimicrobial activity of crude bacteriocin was studied by agar well diffusion method against pathogens. The maximum BLIS activity was observed in *Lactococcus* sp. EF2 strain with the maximum zone of inhibition 1355.88 (AU/ml). Hence EF2 strain was selected for further bacteriocin study. Bacteriocin of EF2 was partially purified by dialyzing against 40% Ammonium sulphate solution. Crude bacteriocin and partially purified bacteriocin (PPB) were compared in the preservation of seafood (fish). Crude bacteriocin shows better results than PPB (Partially Purified Bacteriocin) as it also contains Organic acid, Aminoacid etc., which inhibits the growth of the enteric pathogen.

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Conflict of interest statement

We declare that we have no conflicts of interest

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